The role of UBE3A in gene regulation in relation to AS.

Background
Mutations affecting ubiquitin-ligase 3a UBE3A (E6-AP) cause Angelman Syndrome (AS). Although most studies have focused on the synaptic function of UBE3A, several recent studies showed that UBE3A is highly enriched in the nucleus of both mouse and human neurons (Burette et al., 2017; 2018; Miao et al., 2013). We have recently elucidated the mechanisms that underlie the differential targeting of UBE3A to the nucleus and cytosol (data presented at ASA meeting; revised manuscript submitted to Nature Neuroscience, from here on referred to as Avagliano Trezza et al., 2019). Most importantly, the cytosolic (synaptic) localization versus the nuclear localization is controlled by the differential expression of a cytosolic isoform and nuclear UBE3A isoform, which only differ at their N-termini. Notably, we found that mice that express exclusively the nuclear UBE3A isoform are indistinguishable from wild-type control mice, whereas mice expressing exclusively the cytosolic UBE3A isoform show all the neurological phenotypes of AS mice (Avagliano Trezza et al., 2019). These results strongly suggest that the critical role of UBE3A is in the nucleus, and not in the cytosol/synapse.

Given the enrichment of nuclear-localized UBE3A within the euchromatin-rich transcriptionally-active regions of the nucleus (Burette et al., 2017; Miao et al., 2013), we hypothesize that UBE3A may be involved in the regulation of gene expression, and that such regulation is critical for AS. Note that the observation that UBE3A is involved in gene regulation was already discovered 20 years ago, as it was shown that UBE3A functions as a cofactor for (steroid) nuclear hormone receptors (Nawaz et al., 1999; reviewed by Ramamoorthy and Nawaz, 2008). Specifically, UBE3A has been shown to co-activate the transcriptional activity of the progesterone receptor (PR), mostly in a ligase-independent fashion (Nawaz et al., 1999), as well as the androgen receptor (AR) (Khan et al., 2006) and the Glucocorticoid receptor (GR) (Godavarthi et al., 2012; Khan et al., 2006). Moreover, UBE3A is involved in the breakdown of Estrogen receptor (ER) (Li et al., 2006; Reid et al., 2003), in regulating the ligand-independent transcriptional activity of Peroxisome Proliferator-Activated Receptor PPARα and PPARβ (which appears to be ligase-dependent, but proteasome-independent) (Gopinathan et al., 2008).

From these aforementioned studies, there is no clear emerging picture how UBE3A is involved in the regulation of these receptors. Like other coactivators for nuclear hormone receptors, UBE3A contains LXXLL motifs, which are thought to be important for receptor interaction.
(Huibregtse et al., 1993; Nawaz et al., 1999). But some of these interactions appear to depend on ligase function and others not. Using chromatin immunoprecipitation (ChIP) assays, UBE3A was observed to be physically associated with the Mc1r (Low and Chen, 2011). Given that UBE3A possesses a Zinc finger domain in UBE3A, and we found that AS-associated missense mutation in this domain causes a severe disruption of Zn-finger integrity and loss of nuclear retention (Avagliano Trezza et al., 2019), this raises the intriguing possibility that UBE3A could even regulate gene transcription through direct DNA binding. More in depth studies are needed to unravel the role of UBE3A in the nucleus, but more importantly, these studies should be highly focused on the relationship with AS.

Experimental strategy, methods and objectives of the project
To test which genes are under control of UBE3A, and how this regulation is achieved, we will start with an RNA-seq sequencing experiment of a human SH-SY5Y neuroblastoma cell line in which we deleted the UBE3A gene. We will compare these cells with cells that express the nuclear UBE3A isoform (through viral transduction), as well as with RNAseq data from cells expressing the nuclear UBE3A isoform with the Zn-finger mutation (causing UBE3A to be cytosolic) and cells expressing (in-active) UBE3A due to a mutation in the catalytic Cysteine residue. This will allow us to establish the bona-fide transcripts that are regulated by (nuclear) UBE3A, and also establish whether this regulation requires UBE3A ligase activity. The identified genes from the neuronal cultures, will be compared to RNAseq data which we will obtain from brains of isoform specific UBE3A mutants that we have generated (Avagliano Trezza et al., 2019).

To identify the DNA sequences UBE3A binds to (either directly or indirectly) we propose to perform whole genome chromatin immuno-precipitation (ChIP) sequencing to examine whether there are identifiable DNA sequences to which UBE3A is observed to bind. We will perform the chromatin immunoprecipitation using mouse hippocampus and a UBE3A antibody, and sequence the precipitated genomic DNA fragments by next-generation sequencing (ChIP-seq). A detailed protocol for ChIP-seq analysis from mouse hippocampus is available (Sailaja et al., 2012) and in-depth experience with ChIP analyses is present in the lab of collaborator Raymond Poot (Engelen et al., 2015). Immunoprecipitated DNA will be sequenced on an Illumina HiSeq2500 platform at our biomics facility headed by dr. Wilfred van IJcken, as described (Soler et al., 2010).

Potential pitfalls and alternative strategies
ChIPseq and RNAseq are fully operational and regularly performed at the Erasmus MC. ChIPseq with UBE3A may however be difficult. Even though ChIPseq experiments with UBE3A have been performed before (e.g. Low and Chen, 2011), it could be that the antibody
is not good enough for genome wide-ChIPseq. In that case, we will use (N-terminal) tagged UBE3A instead, as we know that this tag does not interfere with the localization of UBE3A. If a genome-wide ChIPseq experiment fails to provide useful data, the RNAseq experiments by itself would still provide useful data to examine the role of UBE3A in regulating gene expression, and to assess if this is mediated by its ubiquitin ligase activity, or through a non-ligase dependent function (e.g. through its LXXLL domain).

**Timetable and deliverables**

This project will take 2 years. At the conclusion of this work, we will have identified the genes that are regulated by UBE3A, and possibly have identified DNA sequences to which UBE3A binds. Moreover, we have validated these genes in Ube3a-isoform specific mouse brains.

**Relevance to AS**

This work will provide important insight in the molecular role of UBE3A in neuronal function. Although one could argue that such studies have already been performed for 15 years, and has not resulted in a breakthrough, it is important to note that all these studies focused on the synaptic function of UBE3A. Our latest data (Avagliano Trezza et al., 2019) clearly shows that the most important role of UBE3A is not in the synapse but in the nucleus. Knowing the precise role of (nuclear) UBE3A provides important insight into the pathogenesis of Angelman syndrome and may not only provide potential biomarkers, but also lead to the identification of drugable targets.

**Budget:**

We request €100,000 from ASA:

- **Personnel (€65,000):**
  - Technician: 2 years, 0.5 Fte: €50,000
  - Postdoc: 2 years, 0.1 Fte: €15,000

- **Mice, consumables, reagents: (€20,000)**

- **Other (€15,000)**
  - Biomics facility (RNAseq and CHiPseq analysis) and bioinformatics

**Brief Information on the research institution (incl. existing equipment)**

Our laboratory is part of the ENCORE expertise center for neurodevelopmental disorders, which includes one of the largest AS centers in the World (>200 patients in follow-up). This close collaboration ensures optimal valorization of our results for the patients. Both in clinical terms as well as scientific terms, we have demonstrated over the past 10 years a long-term commitment towards Angelman Syndrome.
The Elgersma molecular laboratory is fully equipped for all standard molecular biological procedures, including separate, dedicated spaces for bacterial, mammalian, and ES/IPSC cell culture (Biohazard level 1 and 2). In addition, we have two lab spaces for electrophysiology; one for field recording and in vivo recordings, and one lab space for whole-cell electrophysiology.

The Erasmus MC animal facility (EDC) is the largest animal facility of The Netherlands. All animals are housed under IVC conditions, which is connected to the research building. The Elgersma laboratory has 2 technicians who are fully dedicated to managing the animal colony, including genotyping. All the animals needed for this project are present as live stock in our colony.

Erasmus MC has an outstanding Biomics facility (http://www.biomics.nl/wordpress/) which can not only provide all standard services but is also available to set-up and develop new protocols together with the researcher (e.g. see (van der Wal et al., 2018)).

Brief information on collaboration partners.
For this project we will be closely collaborating with the laboratory of dr. Ben Distel, a biochemist with extensive knowledge about ubiquitin ligases in general and in particular for UBE3A and prof.dr. Steven Kushner who studies AS iPS cells. Moreover, we will closely collaborate with dr. Wilfred van IJcken who is heading the Erasmus MC Biomics facility, and with dr. Raymond Poot, who has extensive experience in CHiPseq analysis. Last but not least, we will start collaborating with the laboratory of dr. Onno Meijer (Leiden UMC), who is an expert in nuclear hormone receptor regulation.

Cited references


